

## Down Regulated ABP1 (Amiloride Binding Protein) Gene Expression in Phenytoin Treated Albino Rat Testis – RT-PCR Analysis



### Medical Science

**KEYWORDS:** Phenytoin, Amiloride binding protein and Testis

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### ABSTRACT

*Phenytoin is used for grand malepilepsy, psychomotor, focal cortical, status epilepticus, trigeminal neuralgia, cardiac arrhythmia. Studies show that phenytoin directly affect brain regions that mediate sexuality. Phenytoin may cause sexual dysfunction by inducing secondary effects on reproductive hormones. The present study is aimed at the effect of phenytoin induced differential regulation ABP1 gene in albino rat testis. ABP1 (Amiloride binding protein) catalyzes the degradation of compounds such as putrescine, histamine, spermine, and spermidine Required for male and Female reproduction. The albino rats were divided into two groups, control and test. The test group was given 120mgs/kg/body weight of phenytoin orally and equal amount of normal saline was given for the control group. After 45 days with the rat under deep anaesthesia, the testis were removed from the scrotum and stored in liquid nitrogen. The stored specimens of testis of control and tests group were subjected to cDNA microarray analysis. This study showed the differential expression of gene ABP1 in test group when compared with the control group.*

### INTRODUCTION

Phenytoin is used for the treatment of grandma epilepsy, psychomotor, focal cortical, status epilepticus, trigeminal neuralgia and cardiac arrhythmia. It prevents repetitive detonation of normal brain cells by prolonging the inactivated state of the voltage sensitive neuronal Na<sup>+</sup> channel which governs the refractory period of neurons. Phenytoin reduces Ca<sup>2+</sup> influx during depolarization, facilitates GABA response and inhibits glutamate response. It also prevents intracellular accumulation of Na<sup>+</sup> that occurs during repetitive firing. Studies show that phenytoin directly affect brain regions that mediate sexuality. It may cause sexual dysfunction by inducing secondary effects on reproductive hormones, changes the concentrations of sex steroid hormones. Research suggests that phenytoin adversely affect hormone levels by reducing the level of free testosterone which, in turn, reduces sexual desire. In male in reproduction, polyamine expression correlates with stages of spermatogenesis, and polyamines appear to function promoting sperm motility. Some of the polyamines like spermine, spermidine, and putrescine, are essential to male and female reproductive processes and to embryo/fetal development. The ABP1 (Amiloride binding protein) gene Catalyzes the degradation of compounds such as putrescine, histamine, spermine, and spermidine,

### MATERIALS AND METHODS

#### Animal treatment and sample collection:

Male adult albino rats were segregated into control and test groups. The test group was treated with phenytoin 120mgs/kg body weight/day orally for 45 days. Similarly control groups were given equal amount of normal saline. In life study protocols, including animal housing, dosage, sacrifice and tissue harvesting were as per IAEC guidelines. After 45 days the tissue samples from test and control collected in Rnase free tubes and snap frozen in liquid nitrogen. Frozen tissues were stored in RNA later at -70 c until processed for RNA extraction.

#### RNA Isolation and DNA Microarray Hybridization and Analysis:

RNA was extracted from the testis preserved in RNA later using QIAGEN's RNeasy minikit Cat#74104 and checked for purity and concentration.

#### RT-PCR:

The exponential amplification via reverse transcription polymerase chain reaction provides for a highly sensitive technique in which a very low copy number of RNA molecules can be detected. RT-PCR is widely used in the diagnosis of genetic diseases and, semi quantitatively, in the determination of the abundance of specific different RNA molecules within a cell or tissue as a

measure of gene expression.

#### PCR primers:

The Rat primers were manually designed using Gene Runner version 3.05. The primers were validated using one of the samples and amplicon sizes were confirmed using the Bioanalyzer.

#### PCR Assay:

Using the Affinity Script QPCR cDNA synthesis kit (Agilent - Lot# 6144678), 200ng of DNase treated RNA was reverse transcribed to make 25ng/ul of cDNA. Relative quantification by

q PCR was then done using Brilliant II SYBR Green qPCR Master mix (Lot # 6127067). Each sample was run in duplicates for each gene using 25ng input per reaction. The experiment was conducted using Stratagene Mx3005P (Agilent technologies) platform. The relative expression levels of the genes were determined after normalizing with beta Actin (ACTB) as the reference gene by using Delta Ct method. The sequences and length of the primers used are as shown in the Table given below.

#### PCR Thermal Conditions:

PCR consisted of initial denaturation at 95°C for 10 min followed by 40 cycles of 95°C for 30 s, 60°C for 1min, 72°C for 1 min. A melt curve was also performed after the assay to check for specificity of the reaction.

#### Steps for Calculation:

Each sample was run in duplicates for each gene. Ct values for each gene were averaged for replicates of each sample.

Delta Ct (DCT) was calculated by subtracting the average Ct value of the reference gene from the average Ct of the test gene. (Average Ct Gene - Average Ct reference gene)

The Delta Delta Ct (DDCT) was calculated by subtracting the DCT of the control group from the target group [DDCT=(DCT Target- DCT control )]

2<sup>Δ- (DDCT)</sup> CALCULATION was done for each DDCT to yield absolute values. [ Fold Change= (2<sup>Δ- DDCT</sup>) ]

The absolute values are converted into log base 2 values for comparison with microarray data.

### RESULT

#### qRT PCR Analysis.

Phenytoin induced -1.12 folds down regulated ABP1 (Amiloride

binding protein) gene expression was observed in phenytoin treated group when compared with untreated control group.

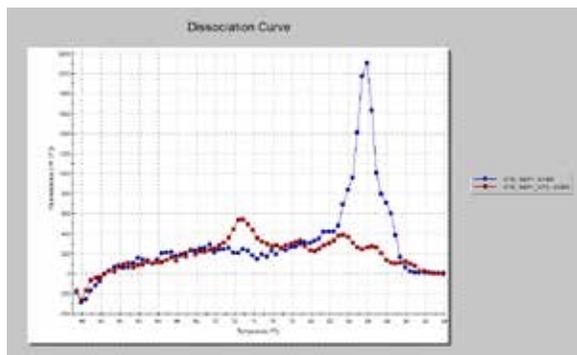
**CONCLUSION:**

ABP1 Catalyzes the degradation putrescine, histamine, spermine, and spermidine, which are essential to male reproductive processes, their absence leads to infertility. when the testicular cells are over stimulated by phenytoin for a prolonged period of time, and the expression of the receptor protein is decreased in order to protect the the testicular cells, the gene product down regulates its own production directly or indirectly, which can result in keeping transcript levels constant proportional to a factor. Down-regulation is a series of actions, changes, or functions resulting in decreased testicular gene and corresponding protein expression.

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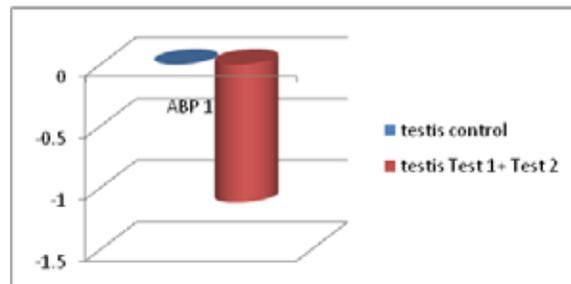
Primer Name	Sequence	Tm	Length	Amplicon size (bp)
Rat_Bactin	AAGGCCAACCGTGAAAAGATGA	64C	22	101
Rat_Bactin	CCAGAGGCATACAGGGACAACA	68C	22	
ABP1_alt_F	TTGAAGATGAGGATTGGTGG	60C	21	164
ABP1_alt_R	CACAGTGTCTCTAGAAGCCA	60C	20	



**Qpcr Fold change data**

Sample/Gene	ABP1	wnt4	b actin
Control	0.00	0.00	0.00
Test 1+Test2	-1.12	4.63	0.00

**Figure1: Bar graph for Qpcr data for ABP1 Gene**



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